From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

...ub. 2005

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 66)

Date of mailing *(day/month/year)*	18.08.2005
Applicant's or agent's file reference	15009pc1
REPLY DUE	**within 1 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK2004/000494	09.07.2004	14.07.2003

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
STATENS SERUM INSTITUT

1. ☒ The written opinion established by the International Searching Authority:
   ☐ is ☒ is not
   considered to be a written opinion of the International Preliminary Examining Authority

2. This first report contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

   When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(e).
   How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.
   Also: For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*. For an informal communication with the examiner, see Rule 66.6. For an additional opportunity to submit amendments, see Rule 66.4.

   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary report on patentability (Chapter II of the PCT) must be established according to Rule 69.2 is: 14.11.2005

Name and mailing address of the international preliminary examining authority:
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WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

International application No.
PCT/DK2004/000494

10/564441

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this opinion is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

   Description, Pages

   1-51 as originally filed

   Sequence listings part of the description, Pages

   1-10 received on 26.10.2004 with letter of 22.10.2004

   Claims, Numbers

   1-23 filed with telefax on 11.02.2005

   Drawings, Sheets

   1/4-4/4 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-23
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY (SEPARATE SHEET)

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: RICH CHANTAL ET AL: "Identification of human enterovirulent Escherichia coli strains by multiplex PCR" JOURNAL OF CLINICAL LABORATORY ANALYSIS, vol. 15, no. 2, 2001, pages 100-103, XP008038376 ISSN: 0887-8013

D2: JP 2003 164282 A (RAKAN:KK; GIFU UNIV) 10 June 2003 (2003-06-10)

D3: EP-A-0 556 504 (SHIMADZU CORP) 25 August 1993 (1993-08-25)

D4: WO 01/94634 A (BIOPOOL INT INC) 13 December 2001 (2001-12-13)

D5: WO 02/36827 A (AUSUBEL FREDERICK M ; GEN HOSPITAL CORP (US); KUDVA INDIRA (US); CALDE) 10 May 2002 (2002-05-10)

D6: WO 03/010332 A (SCHINKINGER MANFRED ; VOLLENHOFER-SCHRUMPF SABINE (AT); FRAENZL GERT () 6 February 2003 (2003-02-06)

D7: WO 95/29261 A (UNIV HAWAII) 2 November 1995 (1995-11-02)

D8: WO 01/48237 A (HOEFT ANDREAS ; STUEBER FRANK (DE)) 5 July 2001 (2001-07-05)

D9: WO 02/053771 A (BIOTECON) 11 July 2002 (2002-07-11)

D10: WO 99/63112 A (FRASER MARK S ; HUNT WESSON INC (US); ROMICK THOMAS L (US)) 9 December 1999 (1999-12-09)

D11: WO 92/17609 A (HOLMES MICHAEL JOHN ; DYNAL AS (NO)) 15 October 1992 (1992-10-15)

D12: WO 00/61720 A (NERENBERG MICHAEL I ; EDMAN CARL F (US); METHA PRESHANT P (US); NANOGE) 19 October 2000 (2000-10-19)

D13: DE 101 23 183 A (BECTON DICKINSON CO) 22 November 2001 (2001-11-22)

D14: WO 00/29618 A (UNIVERISTY OF VIRGINIA PATENT FOUNDATION) 25 May 2000 (2000-05-25)

D15: PATON A W ET AL: "Direct detection and characterization of shiga toxigenic Escherichia coli by multiplex PCR for stx1, stx2, eae, ehxA, and saa" JOURNAL OF CLINICAL MICROBIOLOGY 2002 UNITED STATES, vol. 40, no. 1, 2002, pages 271-274, XP002304663 ISSN: 0095-1137

D16: WO 01/46477 A (CONAGRA GROCERY PRODUCTS COMPA) 28 June 2001 (2001-06-28)

D17: TOMA C ET AL: "Multiplex PCR assay for identification of human diarrheagenic Escherichia coli" JOURNAL OF CLINICAL MICROBIOLOGY 01 JUN 2003 UNITED STATES, vol. 41, no. 6, 1 June 2003 (2003-06-01), pages 2669-2671, XP002304664 ISSN: 0095-1137

1 Claim 1

D17 is considered the closest prior art for the subject matter of claim 1 and discloses a method for simultaneous detection of diarrheagenic *E. coli* groups EPEC, ETEC, VTEC (these are the strains that comprise a verotoxin or shigatoxin gene, which are in D17 referred to as STEC), and EIEC by testing for the presence of the genes eae, vtx (called stx in D17), ipaH, sta (called est in D17), elt and aggR (Tables 1 and 2). D17 implicitly detects also Shigella via the ipaH gene. The method is based on primers chosen to match several clinical subtypes of the virulence gene. The method is performed as a multiplex PCR which comprises a PCR setup designed to enclose all primer sets in one single reaction, leading to the specific amplification of any given template present (see result in Figure 1).

1.1 Claim 1 differs from D17 in that the presence of strains with the ehxA gene is detected along with the other strains, which are a subgroup of STEC (or VTEC) according to the description.

No particular technical effect appears to be associated with this difference.

The problem solved by claim 1 can therefore be seen as the provision of an alternative target for detecting diarrheagenic *E.coli.*

D15 and D16 disclose the detection of ehxA in conjunction with the detection of other markers as a means to characterise diarrheagenic *E. coli* strains. D15 detects ehxA after a multiplex PCR reaction together with vtx1, vtx2, eae and saa in order to determine if a VTEC strain is more likely to be associated with severe disease (Abstract and Conclusions). In D16 a probe for the enterohemolysin encoding gene of *E. coli*, ehxA, is put on an array together with probes targeted at vtx2, eae and *E. coli* 23S rRNA (page 20; Figure 4). Therefore the use of ehxA as one of the targets for detecting diarrheagenic *E.coli* was already known from the prior art. The person skilled in the art who wanted to use an alternative target for the ones in the assay of D17, would have made an arbitrary selection amongst the targets in the prior art, and one of the possibilities would be to use ehxA as a target. The solution of claim 1 therefore cannot be considered inventive (Article 33(3) PCT).

1.2 Claim 1 additionally differs from D17 in that the screening method incorporates a positive control.
The technical effect of this difference is that an control of the procedure is incorporated. The further problem solved by the subject matter of claim 1 can therefore be seen in the provision of a control in the method.
However, using a positive control for a screening method of detecting bacteria is a well known feature, e.g. in D10 (page 17) a universal 16S rRNA probe is used, in D16 a 23S rDNA probe for E. coli is incorporated. Hence, the inclusion of the feature does not require inventive activity from the person skilled in the art.

Claim 1 is hence not considered inventive.

1.3 Claim 7 and 15

The specification of the probes in claim 7 or 15 does not render the screening method inventive, as nucleic acids with such sequences were already disclosed previously.
D2 discloses a nucleic acid molecule which comprises 18 nucleotides of the probe sequence SEQ ID 27 of Table 7 (SEQ ID No 18). D4 discloses a nucleic acid molecule which comprises 17 nucleotides of the probe sequence SEQ ID 28 of Table 7 (SEQ ID No 178). D9 discloses a nucleic acid molecule which comprises 22 nucleotides of the probe sequence SEQ ID 30 of Table 7 (SEQ ID No 82).
D10 discloses a nucleic acid molecule which comprises 17 nucleotides of the probe sequence SEQ ID 26 of Table 7 (SEQ ID No 27). D11 discloses a nucleic acid molecule which comprises 18 nucleotides of the probe sequence SEQ ID 29 of Table 7 (primer 4). D12 discloses a nucleic acid molecule which comprises 22 nucleotides of the probe sequence SEQ ID 31 of Table 7 (SEQ ID No 45). D13 discloses a nucleic acid molecule which comprises 31 nucleotides which have at least 80% identity to the probe sequence SEQ ID 32 of Table 7 (SEQ ID No 36). D14 discloses a nucleic acid which is a 20 nucleotide part of the probe with SEQ ID No 36 (SEQ ID No 1). Hence, D2, D4, D9-D14 each disclose polynucleotides as in claims 7 or 15, the incorporation of these features does not render the claim to which they refer inventive.

1.4 Claim 2 and 14

Claims 2 and 14 refer to a primer selected from the group consisting amongst others of the primer sequences of table 3 (SEQ ID Nos 1-25).

D2 discloses a nucleic acid molecule according to SEQ ID NO 176 which is identical to a primer with SEQ ID No 2 of Table 3.
D3 discloses nucleic sequences of 20 bp (SEQ ID No 11 and 19) which are parts of the primers with SEQ ID Nos 1 and 7 disclosed in Table 3, D4 discloses a part of 21 nucleotides of the primer with SEQ ID No 13 of Table 3 (Primer Slt1), D5 discloses a nucleic acid comprising a part of 18 nucleotides of the primer with SEQ ID No 14 of Table 3 (SEQ ID No 20). D6 discloses a nucleic acid molecule with a sequence which comprises a 16 nucleotides part of the primer with SEQ ID No 15 of Table 3 (SEQ ID No 7), D7 disclose a nucleic acid which comprises a 19 nucleotides part of the primer with SEQ ID No 17 of Table 3 (SEQ ID No 6), D8 disclose a nucleic acid which comprises a 19 nucleotides part of the primer with SEQ ID No 24 of Table 3 (Primer sknI) and D9 discloses nucleic acids which comprise the primer with SEQ ID 16 of Table 3 (Probes with SEQ ID NO 24,25 and 39). Hence, the documents D3-D8 disclose already primers related to those of claims 2 and 14, hence the inclusion of these features in the method of claim 1 does not render the claim inventive (Article 33(3) PCT).

2 Claims 16 and 17, Claim 23

Several documents disclose nucleic acids which are able to prime or hybridise to the genes ipaH, elt, eae and st (e.g. in D1) or to ehxA, eae and vtx1, vtx2 (e.g. D15). To combine such nucleic acids in a kit cannot be considered inventive (Article 33(3) PCT).